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NEPHROLOGY FORUM

Mechanisms of urine concentration

Principal discussant: ROBERT W. BERLINER

Yale University School of Medicine, New Haven, Connecticut

Preface

In this issue of the Forum we make a modest departure from our usual case-oriented discussion. Because basic scientific principles form the backbone of all pathophysiologic reasoning, we present here a strictly physiologic discussion of the development of the countercurrent hypothesis of urine concentration and dilution. The way in which this theory developed exemplifies several recurrent themes in the history of science: valid hypotheses ignored for years, new observations forced into an outmoded theoretical framework, false pathways traversed because of erroneous experimental data, clinical acceptance of a new hypothesis, and lingering doubts that motivate additional studies forcing refinement of existing "truths."

Clinicians will have no difficulty recognizing the implications of the countercurrent hypothesis. Dilutional states, drug-induced concentrating disorders, and mechanisms of diuretic action are a few of the clinically relevant items that we comprehend better because of it, and we do a better job of treating the sick now that this mechanism is understood.

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Discussion

DR. ROBERT BERLINER (*Dean, Yale University School of Medicine, New Haven, Connecticut*): The mechanism for producing a urine more concentrated than other body fluids is one of the more spectacular adaptations of form to function, at least on the macroscopic scale, in vertebrate physiology. And it is remarkable that for many years the mainstream of renal physiology was totally able to disregard this relationship. Had renal physiologists been a little less parochial, perhaps they would not have missed the fact that studies in comparative anatomy, generally looked down upon then as now, pointed in the right direction almost half a century before the physiologists began to take note of their relevant findings.

In attempting to recount the story of how we arrived at our current understanding of the way in which the mechanism really works, I will try to follow the two paths that finally merged in the late 1950s and begin with the one that seemed to most of us working in renal physiology to be the only one before that time.

The development of renal physiology from the time of the First World War to around 1960 can be associated largely with the names of a small number of outstanding investigators who set the style for the rest: A. R. Cushny, A. N. Richards, E. K. Marshall, Homer Smith, R. F. Pitts. The emphasis throughout most of this period was on the integration of glomerular filtration and the activity of the tubules to determine the rates of excretion of various substances. And throughout much of this period, the emphasis was on substances other than the strong electrolytes. This was a reflection, at least in part, of the fact that the methods for measuring most of the strong electrolytes—chloride being a major exception—were exceedingly laborious. In dealing with the major subjects of the studies of that period—inulin, glucose, paraaminohippurate, urea—it was relatively easy to neglect most of the anatomic features of the kidneys, and the nephron came to be regarded as simply a glomerulus, a proximal convoluted tubule, and a distal tubule, with the latter two joined together by an insignificant connecting segment, much as it is in the amphibian, the subject of the early micropuncture work in Richard's laboratory (Fig. 1). Although it was recognized that the effluent from the various nephrons eventually was gathered together in collecting tubules and collecting ducts, these structures usually were denigrated as having little or no significance. To quote Homer Smith's book of 1951, the major compendium on the kidney at the time: "The cytology of the collecting tubules does not suggest any specialized function other than service as conduits, and they are so treated by almost all writers." Smith did add that there was some evidence that might be interpreted as indicating that the

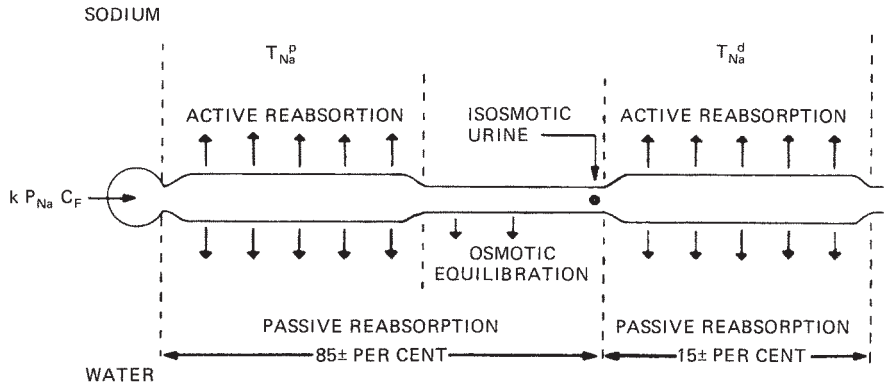

Fig. 1. *"The rectilinear nephron as previously drawn by the writer (H. W. Smith) on several occasions with minor modifications"* [45].

collecting tubules had a reabsorptive function, particularly for water [1].

The ability to form concentrated urine had long been of interest to clinicians because diminished capacity for urine concentration had been recognized as a relatively sensitive indicator of impaired renal function, and the determination of the urinary specific gravity was one of the easiest measurements one could make. For a long time, however, the subject was given little attention by renal physiologists. As attention turned in the late 40s and 50s to the regulation of salt and water excretion, stimulated particularly by the invention of the flame photometer, the formation of dilute and concentrated urines became a matter for explanation. The formation of dilute urine was easily explained by the removal of salt without water from the tubule lumen. The process required only a relatively low permeability to water in the epithelial lining of the tubule from the point of salt reabsorption until the urine left the kidney. Work with the antidiuretic hormone vasopressin had shown that it had the specific property of increasing the permeability of responsive epithelia to water. So there was no great difficulty in providing an explanation for the excretion of dilute urine in water diuresis and the increase of the urine concentration, at least to the point of isotonicity, when vasopressin was given or when an increase in the osmolality of body fluids was imposed so as to cause endogenous release of vasopressin.

The fact that osmolality rose to a value well above that of the blood was another matter, however, and it was considerably more difficult to explain. It was clear that the process involved removal of water rather than the addition of solute, because the concentration of the urine was relatively independent of the nature of the solute that it contained, and it would have been difficult to identify any solute that might have been added to produce the hypertonicity. The only solute whose excretion varied much with urine concentration was urea, and here the change was in the wrong direction, excretion falling as the urine concentration increased. For the same reason, that is, the relative independence of solute excretion from the rate of water excretion, it was generally concluded that the removal of water was a final step in the elaboration of concentrated urine. In any case, we were forced to consider how water might be removed from the urine and transported against what appeared to be a large activity gradient—in other words, by a process of active transport. It was obvious that there were problems involved in the possibility of active water transport that did not apply to the transport of solutes; chief among these were the quantitative considerations. Isotonic fluids are about 55.2 molal with respect to water and less than 0.3 molal with respect to anything else; thus there are, in body fluids, nearly 200 water molecules for every particle of a solute. It would be difficult to imagine that water could be transported one molecule at a time by reversible combination with a carrier—it would require too high a concentration of the carrier molecules, or too high a rate of turnover, or both. So it seemed highly improbable that the process involved what we now would call primary active transport.

Attention was therefore directed to some process that might move water in bulk, the obvious one being something depending on osmosis. A model of such a process had been proposed by Frank and Mayer [2] and although little concern was raised whether this suggested mechanism was feasible, it was generally accepted that some such process could underlie the uphill movement of water. In essence, the Frank–Mayer model invoked the cyclic assembly and disassembly of a polymer within the transporting cell: the polymerization at one margin of the cell presumably decreased osmotic pressure, and the depolymerization at the other surface increased osmolality, thus creating a gradient of osmotic pressure within the cell. Although the model was qualitatively sound, for a time not much attention was paid to its quantitative aspects. For example, the transporting cells would have had to contain an enormous quantity of the organic monomer involved in the process. It was not because of this consideration, however, that the illusion was dispelled that the model could serve the required function. Instead, Brodsky and his associates showed on thermodynamic grounds that the process could not generate a steep enough gradient over the short distance represented by the thickness of the cells that line the tubules [3].

The demise of the only hypothesis that had seemed to provide a plausible explanation for uphill water transport demanded new explorations to determine how the urine was made hypertonic to other body fluids. An entirely different approach to the problem already had been proposed by Wirz and his associates in Basel, but it had been almost totally disregarded. But rather than start in the middle of the story with the work of Wirz, Hargitay, and Kuhn [4], let me go back nearly another 50 years to the origins of their views in the work of the comparative anatomists that I mentioned earlier.

The study usually cited as first indicating that the medulla, and specifically the loop of Henle, had something to do with the

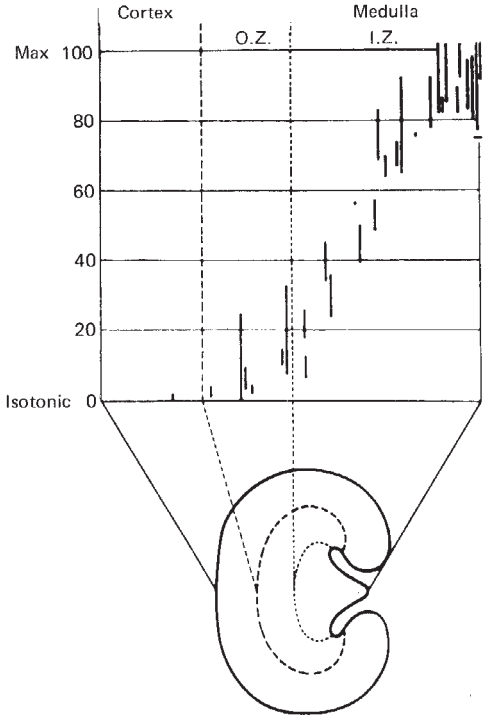

Fig. 2. *Osmolality of fluids at various depths in the rat kidney* [4].

formation of concentrated urine is that of Peter [5], who in 1909 described a correspondence between the length of the loop of Henle in various mammalian species and the specific gravity of the urine that these species produced. In fact there were even earlier studies pointing to the importance of the medulla that might have brought an alert physiologist close to our present views. Filehne and Biberfeld in 1902 had reported that the osmotic pressure of medullary tissue was higher than that of the cortex [6]. Hirokawa in 1908 reported similar findings [7] and wrote (I quote Carl Gottschalk's translation from the original German) [8]:

> The urine present in the medulla has a much higher osmotic pressure than that of the convoluted tubules of the cortex; therefore the osmotic pressure of the urine increases considerably during its passage through the loops of Henle and collecting tubules. . . . the osmotic pressure of the medulla is extraordinarily variable; it is almost without exception higher than that of the cortex, and is higher the more concentrated the excreted urine.

I don't know whether these last two contributions had any influence at the time but, if they did, it had disappeared from the scene by 10 or so years later, and these findings remained more or less unheard of until confirmed much more recently by Wirz and others. Neither of these two papers are among the 2300 cited in Homer Smith's 1951 book [1].

The loop of Henle came back into consideration among students of urine concentration with a paper by Burgess, Harvey, and Marshall in 1933 [9]. In examining the effect of pitressin on representatives of each of the vertebrate classes, they found no effect on urine flow in either catfish or frog but did note a striking effect, often amounting to extended anuria, in the alligator; this effect was associated with what was apparently a very marked fall in glomerular filtration (no mention is made of possible dead-space errors). In the chicken and the dog, they found decreases in urine flow with relatively small changes in glomerular filtration. On the basis of these observations they concluded that antidiuretic hormone had its effect on the loop of Henle, which is the only renal structure present exclusively in birds and mammals, and that its effect was to stimulate water reabsorption. It is interesting that they said nothing about the production of a hypertonic urine, although their conclusions often have been interpreted that way. They did not, in fact, make any measurements of osmolality. Since it has more recently been shown that the frog does respond to vasopressin by producing a less dilute urine [10], the basis for their conclusions was probably incorrect. Nevertheless, their conclusions made a considerably greater impression than did the far more relevant one of Crane four years earlier that only birds and mammals produced hypertonic urine [11].

So for a few years it was believed that hypertonic urine was generated in the loop of Henle. This idea was dealt a fatal blow when Walker, Bott, Oliver, and MacDowell reported the first micropuncture studies in mammals. Three samples collected from rat distal tubules proved not to be hypertonic although the final urine was [12]. In fact, two of the three distal samples appeared to have significantly lower osmolalities than did the blood. At the time nobody knew how to interpret their finding although, of course, it later was recognized as particularly important.

The idea that the loop of Henle might be a countercurrent multiplier first came to the attention of renal physiologists in 1951 with publication of papers by Hargitay and Kuhn [13] and Wirz, Hargitay, and Kuhn [4]. An earlier paper in German by Kuhn and Ryffel published during World War II in the Swiss literature had gone relatively unnoticed [14]. Kuhn, a physical chemist, had developed the idea of the hairpin countercurrent multiplier as a way of carrying out processes of solute concentration. He recognized the hairpin shape of the loop of Henle and, knowing that the loop had been associated with the formation of concentrated urine, suggested that the loop behaved as a countercurrent multiplier. The hypothesis required that the medulla have an increasing osmotic pressure from its base to the tip of the papilla; Wirz, a physiologist, collaborated in an attempt to determine whether such a gradient of osmolality was in fact present [4]. By examining the frozen kidney in polarized light and watching the disappearance of ice crystals as the specimen was rewarmed, they measured the freezing point of the renal structures. Their results indicated that the predicted increase in osmolality was indeed present and that, furthermore, all the structures at the same depth in the renal medulla had the same freezing point (Fig. 2). It is now known that their method failed to detect the relatively small differences in osmolality between the ascending limb of Henle's loop and the other structures. Their findings confirmed those many years earlier of Filehne and Biberfeld [6] and of Hirokawa [7]. The concept of an osmotic gradient was later confirmed by Ullrich and his associates from studies of the osmometric behavior of kidney slices and the finding of increasing concentrations of measured solutes at increasing depth within the medulla [15]. The latter findings of course have been confirmed by many investigators since.

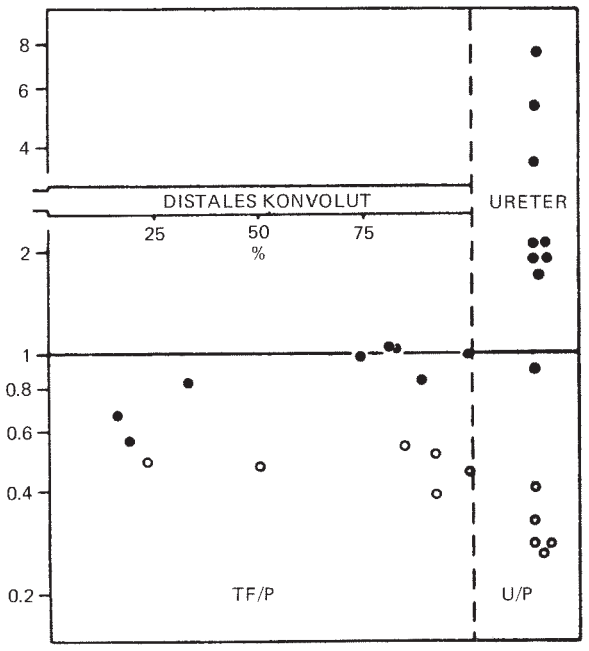

Fig. 3. *Osmolality of fluid from distal tubule* [17].

In a series of continuing studies, Wirz made additional observations that were in conformity with the requirements of the countercurrent multiplier hypothesis. He found that blood obtained from superficial vessels at the tip of the hamster papilla had the same osmotic pressure as the urine [16]. He also confirmed the earlier findings of Walker and his associates [12] that the fluid in the early part of the distal tubule is dilute [17]. He found further that in water diuresis the urine remained dilute throughout the distal tubule, whereas in animals producing concentrated urine, the fluid in later parts of the distal tubule approached, but did not exceed, isotonicity with the plasma (Fig. 3).

One might wonder why, with all these findings in conformity with the countercurrent multiplier hypothesis, acceptance of the model was delayed for such a long time. Aside from the expected resistance to a revolutionary way of thinking about a problem, we may get some idea of a more rational basis for the reluctance to accept the hypothesis if we look at the models that were used to illustrate it.

Figure 4 is from the paper of Hargitay and Kuhn [13]. The three channels in model *b*, the closest to the organization of the renal medulla, may be thought of as the analogues of, from top to bottom, the collecting duct, the descending limb of the loop, and the ascending limb. In this particular model, the driving force for the "single effect" is hydrostatic pressure applied to the central channel against the resistance of the constricted connection between the middle and lower channels, forcing water across the semipermeable membrane, SPM_{12}. This driving force produces an osmotic pressure that increases progressively from left to right in the central and lower channels. At the same time, a much smaller flow through the upper channel, leaving at the right end, is permitted to equilibrate across a semipermeable membrane, SPM_{R1}, and emerges with the same high osmotic pressure as the fluid in the multiplier itself. Although this model was driven by hydrostatic pressure, the authors recognized that the pressures in the kidney were not high enough to drive such a process in the kidney and pointed out that the same change in osmotic pressure could be accomplished by transporting solute across membrane SPM_{12} in the opposite direction, assuming this membrane to be impermeable to water. The advantage of the hydrostatic pressure model was only that the authors were able to construct a mechanical working model. However, there are some problems in considering the model an analogue of the renal medulla. First, it requires that the structures be contiguous to each other, at least for the hydrostatic pressure model. If we use the solute transport mode, we can allow separation of the two limbs of the loop if each transports solute in the right direction, but we cannot separate the collecting duct from one or the other limb because the model requires that the water lost from the collecting duct leave the system in the outflow from the ascending limb. Furthermore, if the water lost from the collecting duct is to enter the loop, the surface of the loop in contact with the collecting duct must have different properties than the remainder of the loop's surface, because the latter must be impermeable to water to prevent the movement of fluid along with the transported solute. Whether we assume water movement from the descending limb to the ascending limb, or solute movement in the reverse direction, the actual situation in the medulla, with the several structures separated by interstitial spaces, indicates that the loop cannot act on the surroundings because of the permeability characteristics that would be required. Thus, it became clear that Hargitay and Kuhn's model, which embodied the best theory of the time, was incomplete.

Davidson and I became interested in the problem of urine concentration as a result of our studies that showed that concentrated urine could be produced in the absence of antidiuretic hormone if the volume of fluid delivered to the concentrating site was sufficiently reduced by constricting the renal artery [18]. This observation led us to question the nature of the concentrating mechanism itself and the possible effects of vasopressin on it. Some features of the Wirz, Hargitay, and Kuhn model were attractive. It provided a function for the loop that corresponded to one that had been suspected for many years and offered an explanation for the adaptation that placed the loop and collecting ducts in isolation from the rest of the kidney. It made the abstraction of water the last step in the elaboration of concentrated urine as had been previously inferred. It explained the dilute character of urine emerging into the distal tubule. It accounted for the high solute concentration throughout the medulla as had then been abundantly demonstrated. And, perhaps most important, it envisioned the active process as salt transport, eliminating the need to invoke active transport of water. In trying to fit all these facts together and yet provide a more plausible function for the loop, Levinsky, Davidson, Eden, and I conceived the idea that the loop should be considered a pumping device delivering sodium salts to the interstitial space of the medulla rather than a countercurrent multiplier [19]. According to this hypothesis, the resulting increase in osmolality of the interstitial space abstracts water from the collecting ducts, and the water from the collecting duct together with the salt pumped in by the loop leaves the medulla via the vasa recta rather than via the nephron. That much of the hypothesis fitted with all the observations. We further suggest-

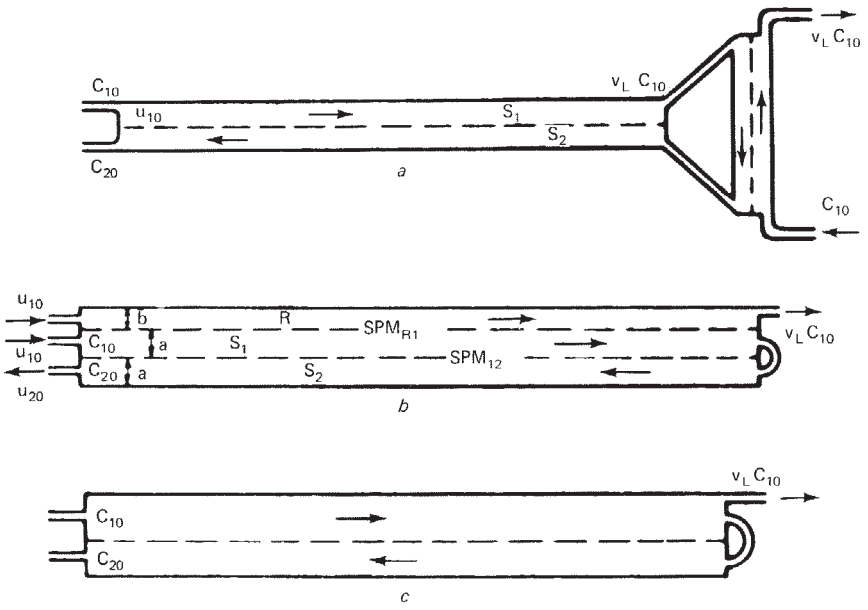

Fig. 4. *Models of countercurrent multiplier* [13].

ed that the loop was impermeable to water throughout its length and became progressively diluted as it pumped out sodium salts. This was not strictly in accord with Wirz's freezing point studies [4], but it was known that he had failed to detect the dilute character of urine in the thick ascending limb and distal tubule, and it seemed not unlikely that the method had failed to reveal the difference between the thin limbs and the rest of the medulla.

A good hypothesis is one that can be shown to be incorrect by a critical experiment. By that standard, our hypothesis was an excellent one because our paper was still in press when Gottschalk and Mylle proved it wrong by showing that fluid collected from the tip of the loop in the hamster papilla had the same high osmolality as did the urine in the collecting ducts and the blood in the vasa recta [8]. The latter was, of course, exactly as predicted by the countercurrent multiplier hypothesis. Had we been a little more imaginative, we would have recognized that our idea of the loop as a pump delivering sodium salts to the renal interstitium did not require abandonment of the countercurrent multiplier. The latter hypothesis required only that the descending limb be permeable to water, solute, or both and that the ascending limb be impermeable to water and be able to pump out salt. That was, of course, the way Gottschalk and Mylle interpreted their findings [8].

There never has been much question since then about the general outline of the mechanism. A few of the predictions that remained untested have since been determined to be accurate. Jamison, Bennett, and I found that the fluid in ascending limbs was more dilute than that in the descending limbs at the same level in the papilla (Fig. 5), the difference being fairly well accounted for by a difference in salt concentration [20]. If any question remained about whether the dilution was caused by loss of solute or by secretion of water (after all, one of the great virtues of the hypothesis was that it eliminated the need to think about active transport of water), Jamison showed that the dilution in the ascending limb was accompanied by a decrease in volume between descending and ascending limbs [21]. An examination of the medullary structures in the rat papilla showed that the permeability to osmotically induced water flow was almost an order of magnitude greater in the descending limb than in the ascending limb and that the permeability was unaffected by vasopressin [22]. The permeability of the collecting duct, on the other hand, was responsive to vasopressin. Similar and much more precise studies of these structures isolated from the rabbit kidney have since been done by Kokko and his associates [23, 24]. I will consider these later when we discuss yet another aspect of this problem.

One prediction from the hypothesis has never been convincingly demonstrated, namely, that the thin ascending limb has the capacity to extrude salt actively. No one has ever questioned the inference that the cells of the thick ascending limb would be able to transport salt actively. Indeed, it has been unequivocally shown by Burg [25] and by Kokko [26] that the isolated thick ascending limb does exactly that, although, contrary to what everyone had previously assumed, chloride rather than sodium is the actively transported ion. Although chloride's being the actively transported ion is important in other respects—particularly as it pertains to the action of diuretics—it is immaterial with respect to the concentrating mechanism. Because the thick ascending limb is limited to the outer medulla, there has not been any difficulty explaining the steep rise in salt concentration in that part of the medulla. The problem has been in accounting for the continuing rise in the inner medulla, which is clearly demonstrable, at least in hydropenic animals (Fig. 6), although the gradient is not as steep as in the outer medulla.

Given the relatively flat epithelium lining the thin limb, and a paucity of mitochondria in the lining cells, investigators always have questioned whether there was sufficient metabolic machinery to support active salt transport at the required rate. A few investigators have detected what might be interpreted as a very small amount of transport against a concentration gradient

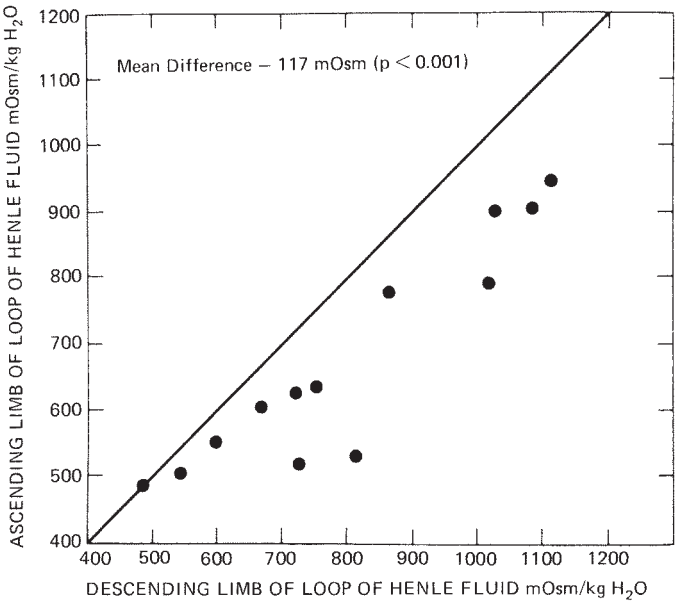

Fig. 5. *Comparison of osmolalities of fluid from ascending limbs and adjacent descending limbs of the loop of Henle* [20].

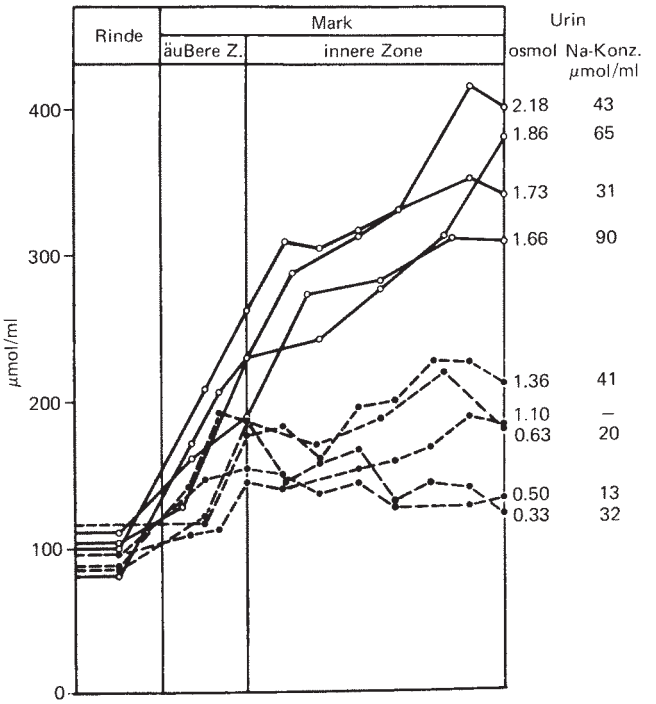

Fig. 6. *Sodium concentration in tissue water of hydropenic* (open circles) *and hydrated* (solid dots) *dogs* [15].

[27, 28], but these findings have never been entirely convincing, and in other studies no evidence suggesting active transport of salt could be found [22, 24, 29]. Consequently there have been a number of attempts to account for the continued rise in salt concentration without invoking any active salt transport by the thin limbs. Several proposals have suggested that the collecting duct might be the site of the requisite salt transport [30, 31], but it is difficult to see how transport of solute out of the urine in the collecting ducts could contribute to making the urine more concentrated. Other investigators have proposed that the only active transport of salt occurs in the outer medulla but that the concentration could continue to rise in the inner medulla simply as a result of countercurrent exchange in the inner medulla [32, 33]. These models were shown to be invalid when Stephenson demonstrated that, in a countercurrent system, no concentration greater than that of the inflow can be achieved *without an input of energy* [34]. I emphasize the phrase "without an input of energy" because Stephenson's proof generally was interpreted to mean without active transport of salt (that is, in the ascending thin limb). There is, in fact, an alternative means of supplying free energy to effect transport out of the thin ascending limb. Stephenson did not have that alternative in mind when he published the proof; rather, 6 or 7 years later he [35] and Kokko and Rector [36] simultaneously and independently proposed that the source of additional free energy is the highly concentrated solution of urea in the collecting ducts which, in turn, is the source of a high concentration of urea in the interstitium of the medulla that surrounds the thin limbs of the loop of Henle.

This use of a solution of a second solute to produce a concentrating effect, not possible in a single solute system without a hydrostatic driving force or active solute transport, can be traced back to the 1942 paper of Kuhn and Ryffel [14]. Figure 7 shows their three-compartment model. It was in connection with this model that Wirz said: "It is questionable that this paper was thoroughly studied by many who quoted it." Compartments A and B, each containing 0.1 M sucrose, are separated by a membrane (M_1) of copper ferrocyanide that is permeable to water but not to solute. Compartment C contains 0.1 M phenol and is separated from B by a rubber membrane permeable to phenol but not to water or sucrose. Phenol diffuses into compartment B until B is 0.1 M with respect to phenol as well as sucrose or, in other words, until B has twice its original osmolality. Water then moves from A to B and raises the osmolality of A to equal that of B, but in A the solute is entirely sucrose at 0.2 M.

The proposals of Kokko–Rector and of Stephenson contain the same basic idea illustrated by this model. Compartment A corresponds to the descending thin limb, compartment B to the medullary interstitium, and C to the collecting duct. Sodium chloride is the solute in place of sucrose, and urea replaces phenol. If we now change the properties of membrane M_1 so that it corresponds to the epithelium of the ascending thin limb, which is assumed to be permeable to salt but not to water, sodium chloride can diffuse *downhill* from A to B, diluting A below the concentration that has been reached when it leaves the descending limb. This increases further the osmolality of the interstitium and causes more water to be removed from the collecting ducts through membrane 2, M_2, here being considered permeable to water and urea but not salt.

These models, then, can explain the countercurrent multiplier function of the thin segment without requiring that the ascending thin limb transport salt by an active process. The free energy is supplied by a concentrated solution of urea. Urea is concentrated by the expenditure of metabolic energy in the

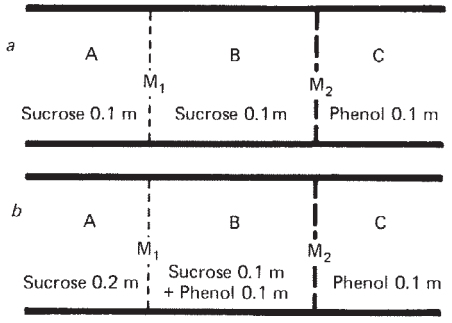

Fig. 7. *Model of Kuhn and Ryffel* [14]. See text.

cortex and outer medulla. In the cortex, salt and water are removed in the distal convoluted and collecting tubules, a process that leaves behind an isotonic solution containing mostly urea. In the outer medulla, urea in the collecting tubule is concentrated further by the movement of water into the medullary interstitium, which is made hypertonic by the active transport of salt by the thick ascending limb. It should be noted that Stephenson did not conclude that salt transport by some active process was absent in the thin ascending limb, but only that if salt transport did occur, it would be more efficient as a consequence of the less unfavorable (or possibly favorable) concentration gradient. In essence, the Kokko–Rector model is a special case of the Stephenson model.

The attractiveness of these models is obvious. Not only do they offer an attractive alternative to the hypothesis of an active transport mechanism in the thin segment of the loop (a mechanism that many thought was unlikely a priori), but they provide a critical role for urea, a role that explains some other old observations that often have been confirmed but never have been adequately accounted for. Gamble and his associates in 1934 studied the volume of urine excreted when various solutes were administered to rats on a low-protein diet and found that when pairs of solutes were administered, the volume in which these solutes were excreted was always the sum of the volumes in which each solute was excreted when given alone, except when urea was one of the solutes [37]. In the latter case, the volume in which the two solutes were excreted was lower than that when the non-urea solute was given alone, yielding what they called "An economy of water in renal function referable to urea." This finding has been confirmed several times [38, 39]. Although some had tried to explain why urea might be excreted largely in water already required for the excretion of other solutes [19], there had not previously been an explanation of the observation that urea could reduce the volume in which other solutes were excreted.

The passive model of the operation of the thin segment of the loop of Henle imposes a number of requirements that are subject to experimental verification. The first of these relates to the permeability of the two limbs. If the system is to work, conditions must be such that a high concentration of urea in the interstitium produces a high concentration of sodium salts in the descending limb. And in the ascending limb, sodium chloride must be able to diffuse out into the interstitium (which is postulated to have a lower salt concentration) and in so doing render the fluid in the lumen more dilute than the surroundings. In other words, the descending limb must have a high permeability to water and a low permeability to both salt and urea, whereas the ascending limb must have a high permeability to salt, a lesser permeability to urea, and a low permeability to water. As I mentioned earlier, the permeability to water in the two limbs had been found generally to conform to these requirements [22]. The characteristics of the two thin limbs from the rabbit kidney were studied in detail by Kokko [23] and by Imai and Kokko [24] by perfusion in vitro. Their findings conform precisely to the model: they found the descending limb to be virtually impermeable to the relevant solutes and highly permeable to water, and the ascending limb to have the opposite characteristics.

Given that this model of a completely passive thin limb explains so many of the known observations, and given also that the permeability characteristics seem so well suited to this function, it would appear that the last piece of the puzzle has been fitted in and that we should be looking elsewhere for problems to work on. Almost, but not quite; a few bothersome facts remain that don't quite conform.

Or perhaps it is the sand rat *Psammomys obesus* that does not conform. At least it would be very difficult to explain the findings of de Rouffignac and his colleagues [40] on the basis of an unmodified version of the Kokko–Rector model. First of all, psammomys doesn't seem very dependent on urea for producing a highly concentrated urine. This animal, with the most highly developed thin segments of the loop of Henle of any animal so far encountered, seems able to produce a concentrated urine without much urea; osmolalities over 2000 mOsm have been obtained with urines containing only a little over 100 mM urea [40]. In most other animals, such a low concentration of urea would be associated with a marked lowering of the maximum concentration of the urine. Even more important is a number of observations suggesting that attainment of the high concentration of solute in the descending limb is largely attributable to salt entry rather than to water loss. Suggestive of the latter is the fact that the degree to which inulin is concentrated at the tip of the loop (a measure of water loss) is poorly related to the extent to which the osmolality is increased [40, 41]. Perhaps even more difficult to fit with the passive mechanism is the relationship between the amount of sodium that reaches the tip of the loop and the amount of sodium that is filtered. On the average, in two separate studies there was more sodium at the tip of the loop than ordinarily escapes reabsorption in the first half of a superficial proximal tubule and, in more samples than is likely to be attributable to experimental error, more sodium reached the tip of the loop than had been present in the glomerular filtrate of the punctured nephron [40, 41]. Assuming these observations are correct, and I see little reason to doubt that they are, they seem to exclude the fully passive mode for the thin segments. But psammomys is unique in other respects, and perhaps it is an exception among mammals in transporting salt actively in the thin ascending limb.

Imai has extended in vitro perfusion studies of the isolated thin ascending limb to the rat and hamster [29]. The permeability properties were found to be similar to those previously found in the rabbit; again, he was able to detect nothing suggesting active transport. He had difficulty isolating adequate segments of descending limbs and found only that these segments had a high permeability to water. These studies suggest that the rat

and the hamster are similar to the rabbit, but other studies in the rat do not completely support an entirely passive process. Perhaps most important is the concentration of urea in loop fluid relative to the concentration of urea in the medullary interstitium. The hypothesis of a passive process dependent on urea requires that the urea concentration outside the loop exceed that inside, at least to the extent that the salt concentration inside exceeds that in the interstitium, since this difference is the driving force for the mechanism. Jamison and his associates have found that the concentration of urea at the bend of the loop is lower, but not much lower, than the concentration of urea in the collecting ducts, the latter setting an upper bound on the interstitial urea concentration [42]. They also found that the concentration of sodium in the loop fluid exceeds that in the vasa recta by a small margin [43]. The differences, although significant, are small enough to raise a question about whether they are sufficient to account for the passive reabsorption of enough salt. In addition, these differences set a low upper bound on the single effect for the countercurrent multiplier.

Bonventre and Lechene pointed out a somewhat different difficulty with the urea concentration in loop fluid; if the thin segment is to contribute any free water, the urea concentration in fluid at the bend of the loop in the completely passive model cannot exceed the concentration of urea in the interstitial space at the junction of inner and outer medulla [44]. In fact, since the thick ascending limb is virtually impermeable to urea, whatever urea is present at the junction of inner and outer medulla also will be delivered to the distal tubule, so that the concentration of urea at the inner medullary–outer medullary junction also will be found in the distal tubule and will limit the extent to which that fluid can be dilute. In other words, the urea concentration determines the extent to which that nephron generates free water and contributes to the entire concentrating process. It is doubtful that the urea concentration is low enough, at least in the rat, to conform to the requirements imposed by this analysis. Pennell, Lacy, and Jamison have found urea concentrations over 300 mM at the tip of the loop in Sprague-Dawley rats [42]. The urea concentration in the interstitium at the junction of inner and outer medulla is unlikely to be as high as this, and such a nephron, having a lower salt concentration than its environment at the inner medullary–outer medullary junction, will take up salt rather than contribute it to the medulla. The contents of the thin segment, not being more dilute than the surroundings, will not have contributed more solute than water to the inner medulla.

To circumvent the problem created by the relatively high urea concentrations, Bonventre and Lechene proposed elimination of one of the other assumptions of the Kokko–Rector model; they proposed that the thin descending limb does not have the same osmolality as its surroundings at the junction of inner and outer medulla. What is required for the thin limb to contribute excess solute to the inner medulla is that the fluid leaving the inner medulla be more dilute than that entering. This state can be achieved by making the ascending limb contents more dilute or the descending limb contents more concentrated than their environment, and Bonventre and Lechene suggest that the latter is the case. I must confess that I am less than enthusiastic about this proposal. It makes critical the rate at which descending limb fluid attains the same osmolality as its surroundings so that, for example, the figure with which their model is illustrated would permit the inner two-thirds of the inner medulla to make no contribution to the concentrating process [44]. I also have reservations about hypotheses that can't be subjected to experimental test and, at least at this time, there is no way to ascertain whether the postulated hypertonicity of the descending limb at the inner medullary–outer medullary junction actually is present.

This is the point at which one customarily draws things together and reaches some conclusion, but I'm afraid that for now we will have to leave things hanging. Attractive as the passive model of the thin segment is, there are some apparently hard facts that leave it in doubt. We can hope that in time the contradictions will be resolved.

Questions and answers

DR. JORDAN J. COHEN: On at least a couple of occasions during your historic review, you pointed to observations that were published in the literature but virtually ignored. You intimated that had those reports been more widely read, our thinking about urinary concentration might have evolved quite differently. We are now living at a time when Current Contents, Medlars, Citation Indexes, and the like make access even to obscure literature very easy. In your judgment, could such a thing happen now? Are there important observations in the literature that we have ignored for 20 years?

DR. BERLINER: There are two reasons why things get ignored. One, nobody reads them, and two, they read them but don't believe them or it doesn't seem to fit into their way of thinking, so they disregard them. And I think in this case it is more the latter that we were dealing with rather than the former. It is probably true that until Wirz, Hargitay, and Kuhn published their paper, nobody in the medical field had read a paper of Kuhn and Ryffel that was published, not only in German during the war, but also in a physical chemistry journal. That is very likely to be missed by biologists. But, other than that I don't think it was that nobody read the papers. I just think they read them and said, "What am I going to do with that?" and threw them away.

DR. JOSEPH BONVENTRE (*Renal Unit, Massachusetts General Hospital, Boston*): I would like to answer your criticisms regarding our model of the renal concentration process. Our model does account for concentration throughout the inner medulla. This is demonstrated in the numerical illustration of the model that we present in our paper [44]. The collecting ducts play an important role in interstitial concentration in the deeper portions of the inner medulla. The sodium chloride that comes out of the loop of Henle in the upper portion of the medulla results in withdrawal of water from the collecting ducts. You have shown that the reflection coefficient of the collecting duct in the inner medulla is greater for sodium chloride than it is for urea [22]. Since the fluid in the collecting duct is high in urea and low in sodium chloride, while the interstitial fluid has a greater portion of its total solute content made up of sodium chloride, equilibration of effective osmotic pressure across the collecting duct epithelium will result in a higher ideal osmotic pressure in the collecting duct than in the surrounding interstitium. This difference in ideal osmotic pressure accounts for the necessary net solute addition to the inner portions of the inner medullary intersititum.

DR. BERLINER: You can provide urea, but you can't provide any salt then, and it is the salt concentration that I am concerned about. You are quite right. You can get more urea; obviously, that is quite true. And I know that the mass balance equations work. But then the mass balance equations work out in Kokko and Rector's model, and that model doesn't work for exactly the reason that you pointed out: the urea concentration is too high. So, mass balance is not a sufficient condition for establishing that a model will work.

DR. BONVENTRE: The profile in the inner medulla for sodium chloride is certainly much flatter than it is for urea. Our model does provide for salt concentration throughout the inner medulla. As we have demonstrated, active reabsorption of nonurea solute in the collecting duct in the inner medulla together with the net nonurea solute reabsorption from the loops of Henle can readily account for a sodium chloride gradient throughout the inner medulla. Finally, I would agree that mass balance is a necessary but not sufficient condition for establishing that a model will work. The model is testable, however, and attempts are being made to compare solute concentrations in the various compartments of the medulla near the outer–inner medullary junction using techniques of electron microprobe analysis.

DR. BERLINER: Obviously it is much steeper in the outer medulla. When the animal is producing a dilute urine, almost all the increase in salt concentration is in the outer medulla. When it is producing a concentrated urine, the concentration of salt goes up much more steeply in the outer medulla, but it does continue to rise in the inner medulla and that is what I am concerned about. Urea doesn't worry me. It is true that if we have urea on the same scale it is much steeper in the inner medulla and relatively not steep at all in the outer medulla.

DR. COHEN: Dr. Berliner, you noted the striking correlation between form and function for this particular aspect of renal physiology and how the thin ascending limb just doesn't have the requisite form to carry out a heavy active transport function. The present theory rests heavily on differential permeabilities of the descending thin limb versus the ascending thin limb. Are there morphologic correlates that would argue one way or the other about such permeability differences?

DR. BERLINER: Let me put it this way. Whether they are correlates of that particular difference I don't know, but there are differences. It was always said that one couldn't tell the difference between ascending and descending thin limbs, but in more modern work with electron microscopy there are clear differences. Permeability is pretty difficult to see. I don't think anybody has ever detected what it is that changes in making a membrane permeable or impermeable. That is a characteristic of the plasma membranes, and the plasma membranes are pretty thin; they don't appear to have very much in the way of structure. So even if one can't see any morphologic differences between them, the physiologic observations are quite clear; there is no doubt that there is a big difference in the permeability characteristics.

DR. COHEN: What about at the level of embryology? Is there any embryologic reason for thinking that the hairpin turn should demarcate two different sets of anlagen?

DR. BERLINER: Not that I am aware of, but I can't say that I really would know if there were. As a matter of fact, the change occurs almost certainly just a very little bit before the turn. So it's in the descending limb rather than at the tip of the loop. But you would have to ask an embryologist, which I am not.

DR. FRANK EPSTEIN (*Director, Renal Division, Beth Israel Hospital, Boston*): One of the most striking pathologic situations in which this system seems to be disturbed is potassium deficiency. The disturbance is all the more striking since diluting ability is very often preserved and glomerular filtration rate is very often normal. To my knowledge, no one has pinpointed the precise locus of the disturbance but, as the grand master and super thinker of concentrating mechanisms, where would you look if you had to direct an eager investigator?

DR. BERLINER: I would look for somebody else! I think you would have to look at the effects of vasopressin on the membranes under those circumstances because I suspect it's not in the concentrating mechanism itself, but in the permeabilities that expose this mechanism.

DR. JEROME P. KASSIRER: If there is little urea in the highly concentrated urine of sand rat, what solute accounts for the high osmolality? Is it salt?

DR. BERLINER: Yes, it's salt. They live on so-called halophile plants, plants that grow in highly salty soils and are loaded with sodium chloride. They don't have much protein intake to require that they get rid of urea. They do have an enormous intake of sodium chloride, and they probably have a very high potassium intake also. As far as I know, all the studies on them have been done in Paris. When Rex Jamison tried to find out whether he could find the same thing as de Rouffignac did, he very wisely chose to go there instead of having them send him the rats.

DR. KASSIRER: There appears to be a large evolutionary gap in renal structure between animals that do not have countercurrent mechanisms and those that do. Does this remarkable evolutionary jump require some special explanation?

DR. BERLINER: No. Actually, I have always been struck by the fact that it doesn't. All it requires is an overgrowth of that particular part of the nephron. If you just take that connecting segment that is present in the amphibian and attach a hook to it and pull it down, it's all done. If you keep the descending part with characteristics somewhat like those in the proximal tubule and the ascending part with characteristics resembling those in the distal tubule, you have a loop of Henle that behaves just the way you want it to. So, although it is a striking morphologic difference, it's not really an entirely new structure but one that could easily be adapted from what was there before.

Reprint requests to Dr. R. W. Berliner, Dean, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, USA

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